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Table 8. Selection of catalytically active phage-Stoffel particles.

$\phi_i$ [a]	$\phi_f$ [b]	Yield	Conditions [c]
in tu	in tu	in %	
8.4x10 <sup>5</sup>	2.0x10 <sup>4</sup>	2.4	
3.6x10 <sup>5</sup>	1.0x10 <sup>2</sup>	0.028	- primer 1b
4.4x10 <sup>5</sup>	3.0x10 <sup>2</sup>	0.068	- biotinylated dUTP 2
4.8x10 <sup>5</sup>	3.0x10 <sup>2</sup>	0.062	- template 3
4.4x10 <sup>9</sup>	4.0x10 <sup>6</sup>	0.091	- trypsin
1.5x10 <sup>9</sup>	5.5x10 <sup>5</sup>	0.037	- trypsin, - primer 1b

$\phi_i$  and  $\phi_f$  denote the number of transformation units (tu) prior [a] and after [b] the selection. Yield =  $\phi_f / \phi_i$ .

[c]: + primer 1b, + biotinylated dUTP 2, + template 3 and + trypsin.

#### Example 9. Selection for disulphide-containing polypeptides.

For the cloning of (poly)-peptide encoding DNA fragments and their display for selection between barnase and p3, the phage fd-3 is constructed (Fig. 5). Phage fd-3 comprises the H102A mutant of barnase N-terminally fused to the p3 gene of phage fd-TET. Between the codon for the last residue of barnase and the first residue of p3 is the nucleotide sequence *CTG CAG GCG GTG CCG CCG CA*. This sequence contains a PstI DNA restriction site (in italics) for insertion of DNA fragments flanked by PstI restriction sites. The sequence further introduces a frame shift between barnase and p3, which prevents expression of the correct p3 reading frame in fd-3. Phage particles of phage fd-3 therefore do not display the infection protein p3 and are non-infectious.

Phage fd-3 is therefore well suited as a cloning vector as vectors without PstI DNA inserts after ligation are not propagated during selection. Statistically 1 out of 3 random DNA inserts in the PstI restriction site will (except for the presence of stop-codons

See  
7/30/02  
for correction  
to add seq  
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